

PATENT
 Attorney Docket No. 020552—001410US
 [Client Ref. No. 1029.1]

Kip3A

864 aa full length sequence

The motor domain is amino acids 5–348 or base pair 143–1174.

5	GC GG CG CG AAT CGG C ACC AG GGG CG CT CT C CCG GT GT GGG T ACT G CT GT CT GT GGT	60
10	1 GT GG CT GT GGG ACC CG TG AG CA AG CAG CG AC GCC AG CG CG GG AG A ACC GAC GAA AG GT GT	120
15	121 CACC ACAGT GAT GGC AGT GG AGG GACAGC AC GAC GCT GCA AGT AGT GGT AC GGG TG CG G CCCCC Met Ala Val Glu Asp Ser Thr Leu Gln Val Val Val Arg Val Arg Pro Pr	180
20	181 CAC CC CT CGGG AG CT GG AC AG TC AG CGG CGG CC AG TG GT TC AG GT GG TG AC GAG CG GGG T o Thr Pro Arg Glu Leu Asp Ser Gln Arg Arg Pro Val Val Gln Val Val Asp Glu Arg Va	240
25	241 GCT GGT GT TT AAC CCT GAG GAG CCC GAT GG AGG GT TCC CT GG C CT GAA AT GGG GT GGC AC 1 Leu Val Phe Asn Pro Glu Glu Pro Asp Gly Gly Phe Pro Gly Leu Lys Trp Gly Gly Th	300
30	301 CCAT GAT GG CCCC AA AGA AGA AGGG CAA AG AC CT GAC GT TT GT CTT GAC CGG GT CTT GG r His Asp Gly Pro Lys Lys Gly Lys Asp Leu Thr Phe Val Phe Asp Arg Val Phe G1	360
35	361 CGAG GCGGCC ACCCA ACAGG AC GT GT CCAG CAC ACCAC GCAC AG CGT CCT GGAC AG CTT y Glu Ala Ala Thr Gln Gln Asp Val Phe Gln His Thr Thr His Ser Val Leu Asp Ser Ph	420
40	421 CCT CCAG GG CT ACA ACT GCT CAG T GT TT GC CT AC GGG CC ACC GGG GCT GGG AAG AC A CA e Leu Gln Gly Tyr Asn Cys Ser Val Phe Ala Tyr Gly Ala Thr Gly Ala Gly Lys Thr Hi	480
45	481 CAC CAT GCT GGG AAGGG AGGGGG ACC CCGG CAT CAT GT AC CT GAC C ACC GT GGG ACT GT A s Thr Met Leu Gly Arg Glu Gly Asp Pro Gly Ile Met Tyr Leu Thr Thr Val Glu Leu Ty	540
50	541 CAG GCGC CT GGAG GCCC GCC AGC AGG AGA AGC ACT TCG AG GT GCT CAT CAG CT ACC AG GA r Arg Arg Leu Glu Ala Arg Gln Gln Glu Lys His Phe Glu Val Leu Ile Ser Tyr Gln G1	600

Fig. 1A

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GGTGTATAATGAACAGATCCATGACCTCCTGGAGGCCAAGGGGCCCTGCCATCCGCGA
601 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 660
uValTyrAsnGlnIleHisAspLeuLeuGluProLysGlyProLeuAlaIleArgG1
5

GGACCCGACAAGGGGGTGGTGGCAAGGACTTCTTCCACCAGCCAGCCTCAGCCGA
661 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 720
uAspProAspLysGlyValValGlnGlyLeuSerPheHisGlnProAlaSerAlaG1
10

GCAGCTGCTGGAGATACTGACCAGGGGAACCGTAACCGCACGCAGCACCCCCTGATGC
721 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 780
uGlnLeuLeuGluIleLeuThrArgGlyAsnArgAsnArgThrGlnHisProThrAspAl
15

CAACCGACTTCCTCCGCTCCCATGCCATCTTCAGATCTTGTGAAGCAGCAGGACCG
781 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 840
aAsnAlaThrSerSerArgSerHisAlaIlePheGlnIlePheValLysGlnGlnAspAr
20

GGTTCCAGGACTGACCCAGGCTGTCCAGGTGGCCAAGATGAGCCTGATTGACCTGGCTGG
841 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 900
gValProGlyLeuThrGlnAlaValGlnValAlaLysMetSerLeuIleAspLeuAlaG1
25

CTCAGAGCGGGCATCCAGCACCCATGCGAAGGGGGAGCGGCTGCGGAGGGGCCAACAT
901 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 960
ySerGluArgAlaSerSerThrHisAlaLysGlyGluArgLeuArgGluGlyAlaAsnI1
30

CAACCGCTCTGCTGGCGCTCATCAACGTCCTCAATGCCCTGGCCGATGCAAAGGGCCG
961 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1020
eAsnArgSerLeuLeuAlaLeuIleAsnValLeuAsnAlaLeuAlaAspAlaLysGlyAr
35

CAAGACCCATGTGCCCTACCGGGACAGCAAACGTGACCCGCCTGCTCAAAGACTCCCTCGG
1021 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1080
gLysThrHisValProTyrArgAspSerLysLeuThrArgLeuLeuLysAspSerLeuG1
40

GGGCAACTGCCGACAGTGATGATCGCTGCCATCAGCCCCCTCCAGCCTGACCTACGAGGA
1081 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1140
yGlyAsnCysArgThrValMetIleAlaAlaIleSerProSerSerLeuThrTyrGluAs
45

CACGTACAACACCCCTCAAATATGCCGACCGGGGCCAAGGAGATCAGGCTCTGCTGAAGAG
1141 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1200
pThrTyrAsnThrLeuLysTyrAlaAspArgAlaLysGluIleArgLeuSerLeuLysSe
50

CAATGTGACCAGCCTGGACTGTCACATCAGCCAGTATGCTACCATCTGCCAACAGCTCCA
1201 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1260
rAsnValThrSerLeuAspCysHisIleSerGlnTyrAlaThrIleCysGlnGlnLeuG1
55

Fig. 1B

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1261	GGCTGAGGTAGCCGCTCTGAGGAAGAAGCTCCAAGTGTATGAGGGGGAGGCCAGCCCC nAlaGluValAlaAlaLeuArgLysLysLeuGlnValTyrGluGlyGlyGlnProPr	1320
5		
1321	ACCACAGGACCTCCCAGGATCTCCAAGTCGGGACCACCAGAACACCTTCCCAGCTC oProGlnAspLeuProGlySerProLysSerGlyProProProGluHisLeuProSerSe	1380
10		
1381	CCCCTGCCACCCACCCTCCCAGCCAGCCCTGCACCCAGAGCTCCCTGCAGGGCCTAG rProLeuProProHisProProSerGlnProCysThrProGluLeuProAlaGlyProAr	1440
15		
1441	AGCCCTTCAAGAGGGAGAGTCTGGGGATGGAGGCCAGGTGGAGAGGGCCATGGAAGGGAA gAlaLeuGlnGluGluSerLeuGlyMetGluAlaGlnValGluArgAlaMetGluGlyAs	1500
20		
1501	CTCTTCAGACCAGGAGCAGTCCCCAGAGGATGAGGATGAAGGCCAGCTGAGGAGGTTCC nSerSerAspGlnGlnSerProGluAspGluAspGluGlyProAlaGluGluValPr	1560
25		
1561	AACCCAGATGCCAGAGCAGAACCCCACACATGCACTGCCAGAGTCCCCTGCCCTGACCT oThrGlnMetProGluGlnAsnProThrHisAlaLeuProGluSerProArgLeuThrLe	1620
30		
1621	GCAGCCCCAGCCAGTCGTGGGCCACTTCTCAGCACGGGAACCTGGATGGGACCGTTCTAA uGlnProLysProValValGlyHisPheSerAlaArgGluLeuAspGlyAspArgSerLy	1680
35		
1681	GCAGTTGGCCCTAAAGGTGCTGTGCGTTGCCAGCGGCAGTACTCCCTGCTCCAAGCAGC sGlnLeuAlaLeuLysValLeuCysValAlaGlnArgGlnTyrSerLeuLeuGlnAlaAl	1740
40		
1741	CAACCTCCTGACGCCGACATGATCACAGAGTTGAGACCCCTACAGCAGCTGGTGCAAGA aAsnLeuLeuThrProAspMetIleThrGluPheGluThrLeuGlnGlnLeuValGlnG	1800
45		
1801	GGAAAAAAATTGAGCCTGGGCAGAGGCCCTGAGGACTTCAGGCCTGCCAGGGGGCACC uGluLysIleGluProGlyAlaGluAlaLeuArgThrSerGlyLeuAlaArgGlyAlaPr	1860
50		
1861	TCTGGCTCAGGAGCTGTGTTCAAGAGTCAATCCCTGTGCCGTCCCTCTGCCAGAGCC oLeuAlaGlnGluLeuCysSerGluSerIleProValProSerProLeuCysProGluPr	1920
55		

Fig. 1C

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1921	TCCAGGATACACTGGCCCTGTGACCCGGACTATGGCGAGGCAGTGAGTGGCCCCCTGCA oProGlyTyrThrGlyProValThrArgThrMetAlaArgArgLeuSerGlyProLeuHi	1980
5		
1981	CACCTGGGAATCCCGCCTGGACCCAAGTCACCCCAGCCCAGGGTCCGATGGCCCAT sThrLeuGlyIleProProGlyProAsnCysThrProAlaGlnGlySerArgTrpProMe	2040
10		
2041	GGAGAAGAAGAGGAGGAGACCAAGCGCCTGGAGGCAGACAGTCCATGGCCTCAAAGCG tGluLysLysArgArgProSerAlaLeuGluAlaAspSerProMetAlaSerLysAr	2100
15		
2101	GGGCACCAAGGCCAGCGCCAGTCCTCCTGCCCTGCCTAAAGGAGAGGGTCTGCCTGA gGlyThrLysArgGlnArgSerPheLeuProCysLeuArgArgGlySerLeuProAs	2160
20		
2161	CACCAACCTTCACAGGGGCCAGCACCCCAAAGGAGAAAGGGCCTCCTCCCCCTGCCA pThrGlnProSerGlnGlyProSerThrProLysGlyGluArgAlaSerSerProCysHi	2220
25		
2221	TTCCCTCGCGTTGCCAGCCACAGTCATCAAAAGCCGGGTGCCCTGGGCCCTTCCGC sSerProArgValCysProAlaThrValIleLysSerArgValProLeuGlyProSerAl	2280
30		
2281	CATGCAGAACTGCTCCACCCGCTGGCTCTGCCACTGAGACCTCAATGCCACCTTGA aMetGlnAsnCysSerThrProLeuAlaLeuProThrArgAspLeuAsnAlaThrPheAs	2340
35		
2341	TCTCTTGAGGAGCCTCCCTCAAAGCCCAGTTCCATGAATGCATTGGCTGGACAAAAT pLeuSerGluProProSerLysProSerPheHisGluCysIleGlyTrpAspLysIl	2400
40		
2401	ACCCCAGGAGCTGAGCAGGCTGGACCAGCCCTTCATCCCCAGGGCACCTGTGCCCTGTT eProGlnGluLeuSerArgLeuAspGlnProPheIleProArgAlaProValProLeuPh	2460
45		
2461	CACCATGAAGGGCCCAAGCCAACATTTCCCTCCCTGGACCTCTGCCTGCAAGAAGAA eThrMetLysGlyProLysProThrSerSerLeuProGlyThrSerAlaCysLysLysLy	2520
50		
2521	GCGCGTTGCGAGTTCCTCAGTCTCCATGGCGCAGCCGATGCCGCCCTCCCCAGCAG sArgValAlaSerSerSerValSerHisGlyArgSerArgIleAlaArgLeuProSerSe	2580
55		

Fig. 1D

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2581	CACTTTGAAGAGGCCAGCTGGCCCTTGTACTCCCAGAGCTGCCCTGAGTCCCCTGTG rThrLeuLysArgProAlaGlyProLeuValLeuProGluLeuProLeuSerProLeuCy	2640
2641	CCCTAGCAACCGGAGGAATGAAAGGACCTCATCAGGGTGGGAGAGCGCTCTCAGCAGG sProSerAsnArgArgAsnGlyLysAspLeuIleArgValGlyArgAlaLeuSerAlaG1	2700
2701	GAACGGCGTCACCAAGGTGTCCTGACCGCCAGAATGTCCTGACCACCAAGGTGTCCTAAC yAsnGlyValThrLysValSer	2760
2761	CTACCGGCCCCCTGCTGGATACCCCTCTGGACCTGTAGCCACCTGCACCAAGGAGCTGG	2820
2821	ACCTGCCTTCCTTACCTGGGAGCAATTAGTGCCAACACACCTTGCTGTATTAACATCCC	2880
2881	TCCCCAGACATCCATCCTGCTACTCACCCCTGTAAATCTCCTGTTACACTCAGCTTCTT	2940
2941	GGCATGTACATATTCAATTGTGAGTGTAAATGTGCTGCTGTTTTGTTTTGGTGGTT	3000
3001	TTTGTTTTTGTTTTGTTTGAGATGGAGTCTACTCTGTCGCCAGGCTGGAGTG	3060
3061	CAGTGGTACGATCTGGCTACTGCAACCTCCGCCCTGGTTCAAGTAATTCTCCTGC	3120
3121	CTCAGCTTCCAAGTAGCTGGATTACAGGCACCCATACCCACACCCAGCTAATTTCGT	3180
3181	CTTTTTAATAGAGAGGGGGTTTCCATGTTGCCAGGCTGGTCTTGAACCTGACCTC	3240
3241	AGGTGATCCGCCCTGCCTCAGCTTCCAAAGTGCTGAGATTACAGGCATGAGCTACCACGC	3300
3301	CTGGCCCGTGTGCTGTTAAAGGTGCTGCCATGTTCCCCCATCTTTTTTTGAG	3360

Fig. 1E

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	ATGGAGTCTCGCTCTGTCGCCAGGCTGGAGTGCAGTGGTGGCGATCTGGCTCACTGCA	
3361	-----+-----+-----+-----+-----+-----+	3420
5		
3421	AGCTCCGCCCTCCCAGGTTCACACCATTCTCCTGCCTCAGCCTCCCAAGTAGCTGGGACTA	3480
10		
3481	CAGGCGCCCACCAACCACGCCGGCTAATTGGTATTTAGAGATGGGTTCAC	3540
15		
3541	CGTGTAGCCAGGCTGGTCTCGATCTGACCTCATGATCCACCCGCCTCGGCTCCAAAG	3600
20		
3601	TGCTGGGATTACAGGCGTGAGCCACTGGGCCGGCCTCCCTCTCATTATGATGCCCTC	3660
25		
3661	TGTGCAGGCAGACGGCTCTGGCTCTTCCCCACCTGTCTCTAACACAGGCCAACGG	3720
30		
3721	TGATGCCACAGGCAGTAGAGGAGGAATGAGGATGGTTGGGAGCGGGAGTCGCGGCT	3780
35		
3781	TGGCTCTCCTGGTTCTGAGAGGGACATCTCATCCACTCCCCTGGTCCCCAACCA	3840
40		
3841	CAGTCCTGGTGAAGATGGATGATAATGGTGCCTTGATTCCAATGAAGACAGCTTA	3900
45		
3901	TTGCTTAACTCTATTGTACATAGGATACACGTTCACTGTAAAATAAAGTGAAAGGGAA	3960
50		
3961	TTCAGGCTTAATGCTGCACCTAGATATAATGCTAATGATACTGGTTATAGCCTCT	4020
4021	GATCCTTATTCCTGCATATATATAGATATACATATATTGGTATAACAATAAAA	4080
4081	CCGTCTCCATCCTGGAAAAAAAAAA	4108

Fig. 1F.

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DNA sequence of HsKIP3A, motor only

1 GACAGCACGC TGCAAGTAGT GGTACGGGTG CGGCCCGCCA CCCCTCGGGA GCTGGACAGT
5 61 CAGCGCGGC CAGTGGTTCA GGTGGTGGAC GAGCGGGTGC TGGTGTAA CCCTGAGGAG
121 CCCGATGGAG GGTTCCCTGG CCTGAAATGG GGTGGCACCC ATGATGGCCC CAAGAAGAAC
181 GGCAAAGACC TGACGTTGT CTTTGACCGG GTCTTGGCG AGGCGCCAC CCAACAGGAC
241 GTGTTCCAGC ACACCACGCC CAGCGCTCG GACAGCTTC TCCAGGGCTA CAACTGCTCA
301 GTGTTTGCT ACGGGGCCAC CGGGGCTGGG AAGACACACA CCATGCTGGG AAGGGAGGGG
361 GACCCCGGCA TCATGTACT GACCACCGTG GAACTGTACA GGCCTGGA GCCCCGCCAG
421 421 CAGGAGAAGC ACTTCGAGGT GCTCATCAGC TACCAAGGAGG TGTATAATGA ACAGATCCAT
481 GACCTCCTGG AGCCCAAGGG GCCCCCTGCC ATCCCGAGG ACCCCCACAA GGGGGTGGTG
541 GTGCAAGGAC TTTCTTCCA CCAGCCAGCC TCAGCCGAGC AGCTGCTGG AATACTGACC
601 AGGGGAAACC GTAACCGCAC GCAGCACCCC ACTGATGCCA ACGCGACTTC CTCCCGCTCC
661 661 CATGCCATCT TCCAGATCTT TGTGAAGCAG CAGGACCGGG TTCCAGGACT GACCCAGGCT
721 GTCCAGGTGG CCAAGATGAG CCTGATTGAC CTGGCTGGCT CAGAGCGGGC ATCCAGCACC
781 CATGCGAAGG GGGAGCGGCT GCGGGAGGGG GCCAACATCA ACCGCTCTCT GCTGGCGCTC
841 ATCAACGTCC TCAATGCCCT GGCGATGCA AAGGGCCGCA AGACCCATGT GCCCTACCGG
901 GACAGCAAAC TGACCCGCT GCTCAAAGAC TCCCTCGGGG GCAACTGCCG CACAGTGATG
961 961 ATCGCTGCCA TCAGCCCCCTC CAGCCTGACC TACGAGGACA CGTACAACAC CCTC

20

Fig. 2

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Protein sequence of HsKIP3A, motor only

1 DSTLQVVVRV RPPTPRELDS QRRPVVQVVD ERVLVFVNPEE PDGGFPGLKW GGTHDGPKKK
61 GKDLTFVFDR VFGEAATQQD VFQHTTHSVL DSFLOGYNCS VFAYGATGAG KTHTMLGREG
5 121 DPGIMYLTTV ELYRRLEARQ QEKHFEVLIS YQEJVNEQIH DLLEPKGPLA IREDPDKGVV
181 VQGLSFHQPA SAEQLLEILT RGNRNRTQHP TDANATSSRS HAIFQIFVKQ QDRVPGLTQA
241 VQVAKMSLID LAGSERASST HAKGERLREG ANINRSLLAL INVNLNALADA KGRKTHVPYR
301 DSKLTRLKD SLGGNCRTVM IAAISPSSLT YEDTYNTL

10 Fig. 3

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Figure 4:

MAVEDSTLQVVVRVRPPTPRELDSQRGPVQVVDERVLVFNPPEPDGGFPGLKWGGT
HDGPKKKKGKDLTFVFDVFGEAATQQDVFQHTTHSVLDSFLQGYNCSVFAYGATGAG
KTHTMLGREGDPGIMYLTTVELYRRLEARQQEKHFEVLISYQEYVNEQIHDLLPKG
5 PLAIREDPDKGVVQQLSFHQPASAEQLLEILTRGNRNRTQHPTDANATSSRSHAIF
QIFVKQQDRVPGLTQAVQVAKMSLIDLAGSERASSTHAKGERLREGANINRSLLALI
NVLNALADAKGRKTHVPYRDSLKLTRLLKDSLGGNCRTVMIAAISPSSLTYEDTYNTL
KYADRAKEIRLKGNNSKLEGKPIPNPLLGLDSTRTGHHHHHH

10 Grayed sequence is derived from the vector (pCRT7/CT, Invitrogen) and contains a V5 epitope and polyhistidine tag.

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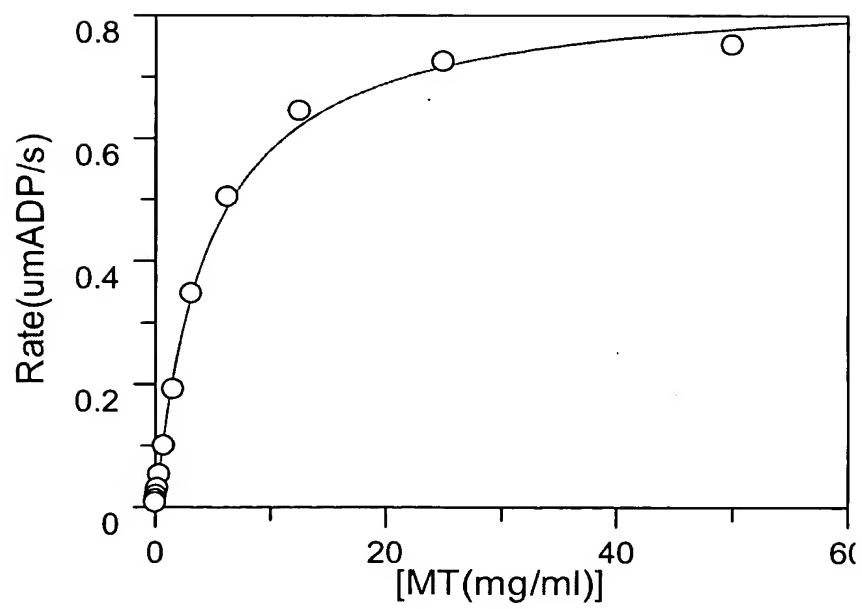
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Figure 5: Nucleotide sequence of the Kip3a fragment used in the ATPase assay

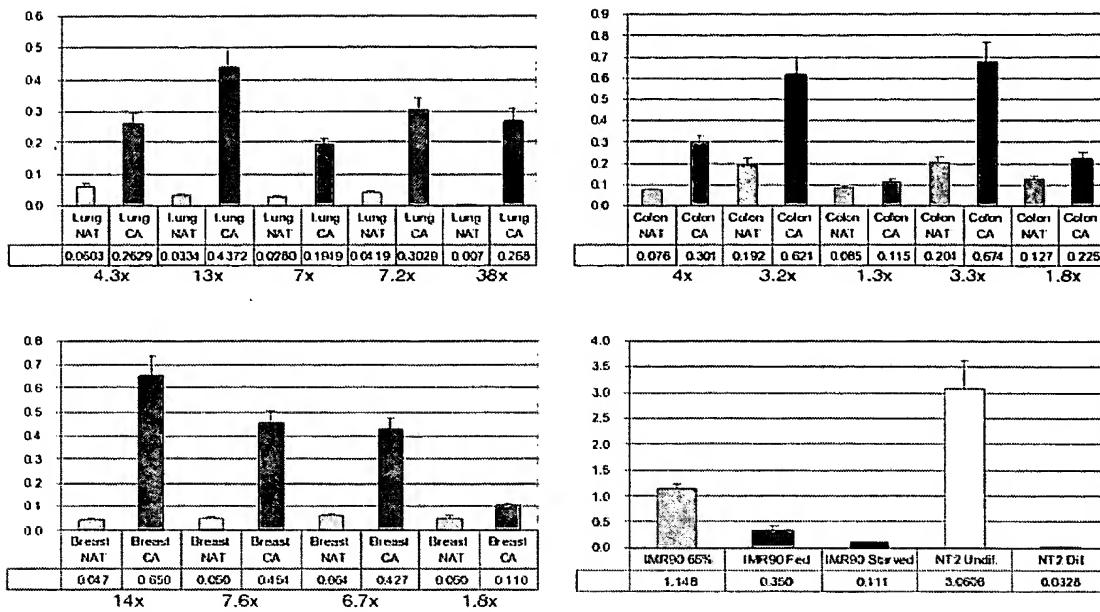
ATGGCAGTGGAGGACAGCACGCTGCAAGTAGTGGTACGGGTGC GGCCCCCACCCCT
CGGGAGCTGGACAGTCAGCGCGGCCAGTGGTTCAGGTGGTGGACGAGCGGGTGCTG
5 GTGTTAACCTGAGGAGCCCAGTGGAGGGTCCCTGGCCTGAAATGGGGTGGCACC
CATGATGGCCCCAAGAAGAAGGGCAAAGACCTGACGTTGTCTTGACCGGGTCTT
GGCAGGGGGCACCCAAACAGGACGTGTTCCAGCACACCACGCACAGCGTCCGGAC
AGCTTCCTCCAGGGCTACAAC TGCTCAGTGTGCTACGGGGCACCGGGCATCATGTACCTGACC
10 AAGACACACACCATGCTGGAAAGGGAGGGGACCCGGCATCATGTACCTGACC
GTGGAAC TGACAGGCGCTGGAGGCCCCAGCAGGAGAACGACTTCGAGGTGCTC
ATCAGCTACCAGGAGGTGTATAATGAACAGATCCATGACCTCCTGGAGGCCAAGGGG
CCCCTGCCATCCCGAGGACCCGACAAGGGGTGGTGGTCAAGGACTTCTTC
CACCAAGCCAGCCTCAGCCGAGCAGCTGCTGGAGATACTGACCAGGGGAACCGTAAC
15 CGCACGCAGCACCCACTGATGCCAACCGCACTCCTCCGCTCCATGCCATCTTC
CAGATCTTGTGAAGCAGCAGGACCGGGTTCCAGGACTGACCCAGGCTGTCCAGGTG
GCCAAGATGAGCCTGATTGACCTGGCTGGCTCAGAGCGGGCATCCAGCACCCATGCG
AAGGGGGAGGGCTGCGGAGGGGCAACATCAACCGCTCTGCTGGCGCTCATC
AACGTCCCTCAATGCCTGGCCATGCAAAGGGCGCAAGACCCATGTGCCCTACCGG
GACAGCAA ACTGACCCGCCTGCTCAAAGACTCCCTGGGGCAACTGCCGCACAGTG
20 ATGATCGCTGCCATCAGCCCCTCCAGCCTGACCTACGAGGACACGTACAACACCC
AAATATGCCGACCGGGCCAAGGAGATCAGGCTCAAGGGCAATTGAAAGCTGAAGGT
AAGCCTATCCCTAACCTCTCCTCGGTCTCGATTCTACCGTACCGGTATC
CATCACCATATTGA

25 Grayed sequence is derived from the vector (pCRT7/CT, Invitrogen) and contains a V5 epitope and polyhistidine tag.

Fig. 6



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5 Fig. 7

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